

The following examples further illustrate the invention but are not to be construed to limit the scope thereof:

TABLE 1. Is a list of clones isolated from banana pulp and the corresponding sequence identity number as provided in the sequence listing herein. The table also illustrates the approximate clone size, the percentage identity and, where relevant, nucleotide similarity with published sequences, based on the results obtained from comparisons with the EMBL sequence database. The table also provides, where relevant, the gene identity of those published sequences and their database accession numbers.

FIGURE 1. Plant transformation vector pUN, containing the UBI polyubiquitin promoter.

FIGURE 2. Plant transformation vector pSHYN, containing hygromycin resistance gene for selection of transformed plants.

FIGURE 3. Plant transformation vector pAN, containing the banana ACC oxidase promoter.

EXAMPLE 1

Construction of a cDNA library of ripening genes

1.1 Isolation of messenger RNA

Total RNA was isolated from ripening (24 hours after ethylene treatment) banana pulp (*Musa acuminata* cv. Grand Nain) as described by Chang et al. Plant Molecular Biology Reporter. Vol. 11(2) 113-116 (1993). Messenger RNA was isolated from total RNA by Oligo(dT)-cellulose chromatography according to Bantle et al., Analytical Biochemistry 72, 413-427 (1976).

1.2 Synthesis of cDNA and Cloning into Vector

The first and second strands of the cDNAs were synthesised from the messenger RNAs using a commercial cDNA synthesis kit (Catalog No. 200450, ZAP Express™ Gold Cloning kit, Stratagene Ltd, Cambridge, Cambs, UK). Double stranded cDNAs were cloned into the ZAP Express™ vector, packaged, mixed with plating bacteria to determine titre and for library screening, following instructions of the suppliers protocol.

1.3 Screening of the cDNA library from banana pulp.

The unamplified cDNA library from ripening banana pulp was differentially screened using cDNA from unripe and ripening banana peel tissue. A proportion of the library was plated individually at low density and duplicate plaque lifts made onto Hybond N nylon filters (Amersham) according to the manufacturer's instructions. One filter was hybridised to dCTP radiolabelled cDNA from green fruit and the duplicate filter hybridised to dCTP radiolabelled cDNA from ripening fruit. Hybridization's were at high stringency. Plaques hybridising preferentially with ripening or green radiolabelled cDNA were picked and re-plated for a second round of selection by differential screening. These clones were numbered as ripening up- or down-regulated peel clones. The clones were in-vivo excised from the ZAP express™ vector into the pBK-CMV phagemid vector using the ExAssist™ interference-resistant helper phage, following instructions from manufacturers protocol.

1.4 Characterisation of the ripening pulp cDNA library and the ripening-related clones.

The ripening cDNA library from pulp tissue were prepared with an efficiency of 3.2×10^5 plaque-forming units per microgram of cDNA. The sizes of the inserts in the peel library was 0.4 - 6.7 Kb with a mean size insert of 1.47 Kb.

From the 250 plaques used in the first screen, 73 putative ripening-related clones were obtained. These 73 clones were partially sequenced using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq® DNA polymerase (Applied Biosystems, Warrington, Cheshire, UK) with forward primers specific for the pBK-CMV vector. From these, the following ripening-related clones were selected. Comparisons of these sequences in the EMBL database using GCG ('Wisconsin') software has identified homologies for the clones listed in TABLE 1 below.

EXAMPLE 2

Construction of truncated sense RNA vectors with the maize polyubiquitin promoter.

A vector is constructed using the sequences corresponding to a fragment of the inserts of one of the sequences 1 to 57. This fragment is synthesised by polymerase chain reaction using synthetic primers incorporating BamHI restriction sites suitable for cloning between a

maize UBI polyubiquitin promoter (Christensen et al, 1992, Plant Molecular Biology, 18:675-689) and a nopaline synthase 3'end termination sequences in the vector pUN (Fig. 1.).

The truncated sense expression cassette is excised by digestion with *Ascl*, the ends of the fragment are made flush with T4 polymerase and it is cloned into the vector pSHYN (Fig. 2.) which has been cut with *KpnI* and the ends made flush with Klenow polymerase. pSHYN contains hygromycin resistance gene for selection of transformed plants.

After synthesis of the vector, the structure and orientation of the sequences are confirmed by DNA sequence analysis.

EXAMPLE 3

Construction of truncated sense RNA vectors with a fruit enhanced promoter.

The 1386bp *HindIII* fragment containing the banana ACC oxidase promoter (UK.

Application No. 9607700.3) is cloned the *HindIII* site in pMSC2 (Fig. 3.) to give the vector pAN.

A vector is constructed using the sequences corresponding to a fragment of the inserts of one of the sequences 1 to 57. This fragment is synthesised by polymerase chain reaction using synthetic primers incorporating *BamHI* restriction sites suitable for cloning between a maize UBI polyubiquitin promoter (Christensen et al, 1992, Plant Molecular Biology, 18:675-689) and a nopaline synthase 3'end termination sequences in the vector pAN.

The truncated sense expression cassette is excised by digestion with *Ascl*, the ends of the fragment are made flush with T4 polymerase and it is cloned into the vector pSHYN (Fig. 2.) which has been cut with *KpnI* and the ends made flush with Klenow polymerase. pSHYN contains hygromycin resistance gene for selection of transformed plants.

After synthesis of the vector, the structure and orientation of the sequences are confirmed by DNA sequence analysis.

EXAMPLE 4

Construction of an over-expression vector with the maize polyubiquitin promoter.